Biochemical Pharmacology, Vol. 55, pp. 1005–1012, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00534-0

Drug Glucuronidation by Human Renal UDP-Glucuronosyltransferases

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Abstract. The UDP-glucuronosyltransferases catalyse the conjugation of glucuronic acid to a wide variety of endobiotics and xenobiotics, representing one of the major conjugation reactions in the conversion of both exogenous (e.g. drugs and pesticides) and endogenous compounds (e.g. bilirubin and steroid hormones). The liver is the major site of glucuronidation, however a number of extrahepatic tissues exhibit particular UDP-glucuronosyltransferase activities. The present study was undertaken to assess the human renal UDP-glucuronosyltransferase system. Enzymatic analysis of human kidney showed that a limited number of UDP-glucuronosyltransferase isoforms were expressed in this tissue. However the level of renal UGT activity towards the anaesthetic propofol was higher compared with human liver. The glucuronidation of propofol is catalysed by UGT1A8/9 suggesting higher levels of this isoform in the kidney. Immunoblot analysis revealed two major UDP-glucuronosyltransferase immunopositive bands to be present in human kidney as compared to four major bands in human liver. The human kidney was capable of conjugating various structurally diverse drugs and xenobiotics. BIOCHEM PHARMACOL **55**;7:1005–1012, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. human, glucuronidation, kidney, biotransformation, xenobiotic, propofol

The liver is generally accepted to be the main contributor to overall drug biotransformation in the body [1]. However many extrahepatic tissues exhibit enzyme activity towards a particular group of compounds. The major tissues of extrahepatic detoxication are those involved in absorption or excretion of chemicals such as lung, kidney and gastrointestinal mucosa [1].

The enzyme UDP-glucuronosyltransferase, UGT,¶ (EC 2.4.1.17) catalyses the transfer of glucuronic acid from UDP-glucuronic acid to a wide variety of endobiotics and xenobiotics. The transfer of the glucuronic acid moiety occurs via a carboxyl, hydroxyl, amino, thiol, or carbonyl group on the acceptor aglycone. The process, glucuronidation represents one of the major conjugation reactions in the conversion of both exogenous (e.g. drugs and pesticides) and endogenous compounds (e.g. steroids and bilirubin) [2–4]. The result is a more polar, water soluble metabolite which can excreted in urine or bile.

The liver is quantitatively the most important site of glucuronidation for the majority of compounds. The overall quantitative contribution of extrahepatic glucuronidation is generally lower than that of the liver, but can play a specific biological role. Rat and bovine olfactory epithelium have been shown to contain a specific UGT isoform which has not been identified in liver or other UGT containing tissues. The enzyme is responsible for the glucuronidation of odorants; the glucuronides produced can no longer stimulate olfactory adenyl cyclase and hence the odorant signal is terminated [5, 6].

The kidney was until recently relatively ignored in drug metabolism studies. However, the kidney receives approximately 25% of the total cardiac output [7] and since all drugs which are excreted in the urine must pass through the kidney this organ may indeed contribute to the total metabolism of drugs in the body. The presence of both phase I and II detoxication enzyme systems in the kidney has been established, primarily within the cells of the proximal tubule, however their significance is less well defined [1, 8].

Studies of renal glucuronidation towards several phenolic compounds, e.g. 1-naphthol and 4-nitrophenol, have shown a significant contribution to the overall metabolism of the compound [2, 9, 10]. Renal UGT activity was found to vary markedly depending on the species and substrate

The nomenclature used in this manuscript is based on published recommendations and is to be defined by the position of the variable exon within the gene complex [45]. The position of the specific isoform formerly known as HlugP4 or UGT1*02 has yet to be resolved. As such, the nomenclature for this isoform will be stated as UGT1A8/9.

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¶ *Abbreviations:* BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BSA, bovine serum albumin; ddH_2O, double distilled water; DMF, dimethylformamide; FK, female human kidney; HKM, human kidney microsomes; HLM, human liver microsomes; MK, male human kidney; NBT, nitro blue tetrazolium; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; UDP, uridine diphosphate; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

Received 7 July 1997; accepted 23 September 1997.

studied [8]. Morphine and testosterone UGT activity are absent from adult rat kidney yet present in rat liver [9, 11]. However, rat kidney is capable of glucuronidating bilirubin [12, 13], an activity which is absent from human kidney [14].

Previous studies of human renal glucuronidation within this laboratory have been limited by tissue availability. The present study was undertaken to determine renal contribution to the glucuronidation of various structurally diverse compounds. In addition, immunoblot analysis was performed to study the renal expression of UGT isoforms.

The human UGT gene family has the potential to encode a large number of UGT isoforms, many of which have been isolated and studied in expression systems. These expression systems have proved invaluable tools in elucidating the substrate specificity of individual isoforms [15]. The analysis of substrates glucuronidated by human kidney may uncover the particular UGT isoforms expressed in human kidney.

MATERIALS AND METHODS

Materials

^{14}C-UDP-glucuronic acid, UDPGA (≥303 Ci/mmol) was obtained from Dupont Ltd. ^{14}C-1-Naphthol (59 mCi/mmol) was purchased from Amersham. Unlabelled UDPGA (triammonium salt) was purchased from Sigma Chemical Company. In addition, nitro blue tetrazolium, (NBT), 5-bromo-4-chloro-3-indolyl phosphate, (BCIP), bovine serum albumin, bilirubin and alkaline phosphatase-labelled anti-goat IgG (whole molecule) were obtained from Sigma. Nitrocellulose (Schleicher & Scheull, BA 85) was obtained from Anderman & Co. Ethylanthranilate was from Kodak. Acrylamide/bis-acrylamide (30%/0.8% (w/v)) was purchased from Scotlab Ltd. All other chemicals and reagent were purchased from Merck-BDH and were of analytical grade.

Human Samples

Human kidney pathology samples were obtained following nephrectomies performed at Dundee Royal Infirmary, with the assistance of Dr. Derek Byrne, Renal Unit. The samples were placed in liquid nitrogen immediately after removal. All the renal tissue used in this study was deemed normal and any potential carcinoma tissue was removed. The majority of the samples were from elderly male patients. Wherever possible, details of medication and diagnosis were obtained for each tissue (Table 1). Human liver was obtained as the result of transplantation, performed within 6 hr post mortem.

Microsomes were prepared as previously described [16] and the protein content determined using bovine serum albumin as standard [17]. Aliquots were stored at −80° and used within 2 months.

TABLE 1. Characteristics of patient tissue samples

Sample	Diagnosis	Medication
MK45	renal carcinoma	unknown
MK68	renal carcinoma	thioridazine
FK78	renal carcinoma polycystic kidney	unknown
MK63(a)	renal carcinoma	atenolol nifedipine ranitidine gaviscon diazepam
MK62	renal carcinoma	bendrofluazide warfarin
MK63(b)	renal carcinoma	no medication
FK47	renal carcinoma	hormone-replacement therapy
MK1	renal carcinoma	unknown
MK2	renal carcinoma	unknown

Thin Layer Chromatographic (TLC) Analysis of Human Renal UGT Activity

The TLC protocol used was adapted from a previously published method [18]. This assay was used to determine UGT activity towards several different substrates in microsomal fractions and cell homogenates. Cell lines heterologously expressing UGT1A1 were assayed as sonicated cell homogenates. Briefly, the conditions were as follows, 100 mM of Tris-Maleate, pH 7.4, 5 mM of $MgCl_2$, 2 mM of UDPGA, (0.2 μCi ^{14}C-UDPGA), 0.5 mM of aglycone and 50–200 μg protein. If reactions were performed with aglycones containing carboxyl groups, an alternative buffer was used (100 mM bis-tris-maleate, pH 6.5) to prevent degradation of any acyl glucuronides formed [19–21]. The time course of the assay was typically 30–60 min and reactions were terminated by the addition of 2 vol of ice cold ethanol.

Proteins were sedimented by centrifugation at 14,000 *g* for 5 min (Eppendorf). The product-containing supernatants were dried down under reduced pressure (Speedevac, Genevac Ltd.), reconstituted in 30 μL of 70% (v/v) ethanol and spotted onto silica plates (Kieselgel 60 F_{254}, 0.25 mm Merck). The solvent system used in this assay was freshly prepared and consisted of butanol:ddH_2O:acetone:glacial acetic acid:30% ammonia (mls 70:60:50:18:1.5). The dried plates were scanned using a digital autoradiograph (Berthold). Typically the radiolabelled glucuronides moved with an R_f value of 0.55–0.65.

Bilirubin UGT Assay

Bilirubin UGT activity was measured by the *O*-ethylanthranilate method described previously [22, 23]. This was performed on samples from human kidney, human liver and a Chinese hamster fibroblast cell line (V79) expressing the human bilirubin UGT isoform UGT1A1 [24].

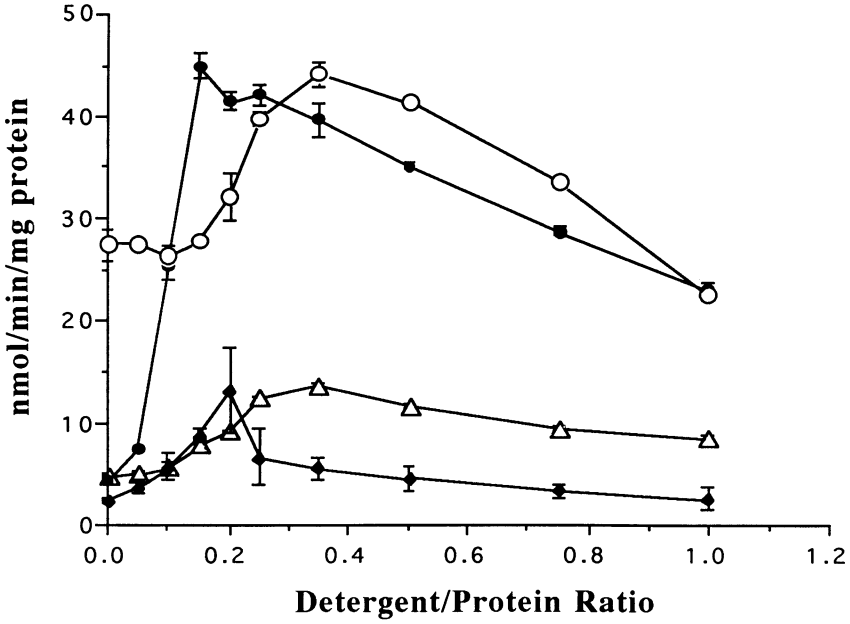

FIG. 1. Effect of lubrol on 1-naphthol UGT activity in hepatic and renal tissues. Assays were performed in duplicate using radiolabelled 1-naphthol. Rat liver microsomes (●); human liver microsomes (○); rat kidney microsomes (◆) and human kidney microsomes (△). Specific activities are the average of three separate experiments ± SEM.

1-Naphthol UGT Assay

The glucuronidation of 1-naphthol was analysed *in vitro* using radiolabelled substrate, 1-naphthol. Unincorporated radiolabelled 1-naphthol was extracted from the reaction mixture with chloroform, based on a previously published protocol [25].

The 1-naphthol UGT assay was also used to determine the degree of latency for each microsomal preparation. Assays were performed [25] in varying amounts of the detergent lubrol PX (12A9). Microsomal proteins (50–100 μg) were preincubated with lubrol on ice for 20 min preceding the assay. Assays were allowed to proceed for exactly 10 min prior to termination and extraction of unincorporated radiolabel.

SDS-PAGE Electrophoresis and Immunodetection of Microsomal Proteins

SDS-PAGE was according to the methods of Laemmli [26]. Fifty μg of protein was typically loaded per lane on a 7.5% polyacrylamide gel. The gel was electrophoresed at 25 mA/gel through the stack and 35 mA/gel through the main resolving section. Following electrophoretic transfer [27] non specific sites on the nitrocellulose were blocked by incubation in 1% (w/v) bovine serum albumin prepared in TBST buffer, (10 mM of Tris, 154 mM of NaCl, 0.05% (w/v) Tween 20, pH 9.0) for 60 min. The nitrocellulose was then gently agitated in primary antibody, an anti-rat liver UGT antibody, [28] diluted 1:2500 in TBST/1% BSA for 60 min followed by 6 × 5 min washes in 1 × TBST buffer alone. The next step was to incubate the nitrocellulose in secondary antibody (anti-goat IgG-alkaline phosphatase, diluted 1:2000) for 30 min followed by 6 washes as above. Prior to development, the nitrocellulose was rinsed for 5 min in 2 changes of developing buffer, (100 mM of Tris, 100 mM of NaCl, 5 mM of $MgCl_2$, pH 9.5). The substrates for colour development with alkaline phosphatase were prepared in dimethylformamide, DMF: 10 mg of NBT was dissolved in 200 μL of 70% DMF and 5 mg of BCIP was dissolved in 100 μL of 100% DMF. Immediately before use these solutions were added to 30 mL of developing buffer. Colour development generally occurred within 10 min. The reaction was stopped by the removal of the developing solution and several rinses in distilled water. The apparent molecular weight of any bands were determined by comparison with standard proteins of known molecular mass.

RESULTS

Drug and Xenobiotic Glucuronidation in Human Kidney Microsomes

The addition of lubrol to the microsomal membrane preparation increased the levels of 1-naphthol UGT activity (Fig. 1). Increasing amounts of lubrol in the membrane preparation ultimately inhibited this activity. This latency has previously been shown to regulate UGT activity at the level of the endoplasmic membrane [29]. Both human and rat kidney were activated approximately 3-fold with the addition of lubrol indicating similar regulation of renal UGT activity between species. The lubrol activation achieved with rat liver was greatest (10-fold). The activation of human liver was not as pronounced (~2-fold) although maximal levels of 1-naphthol UGT activity were similar to rat liver.

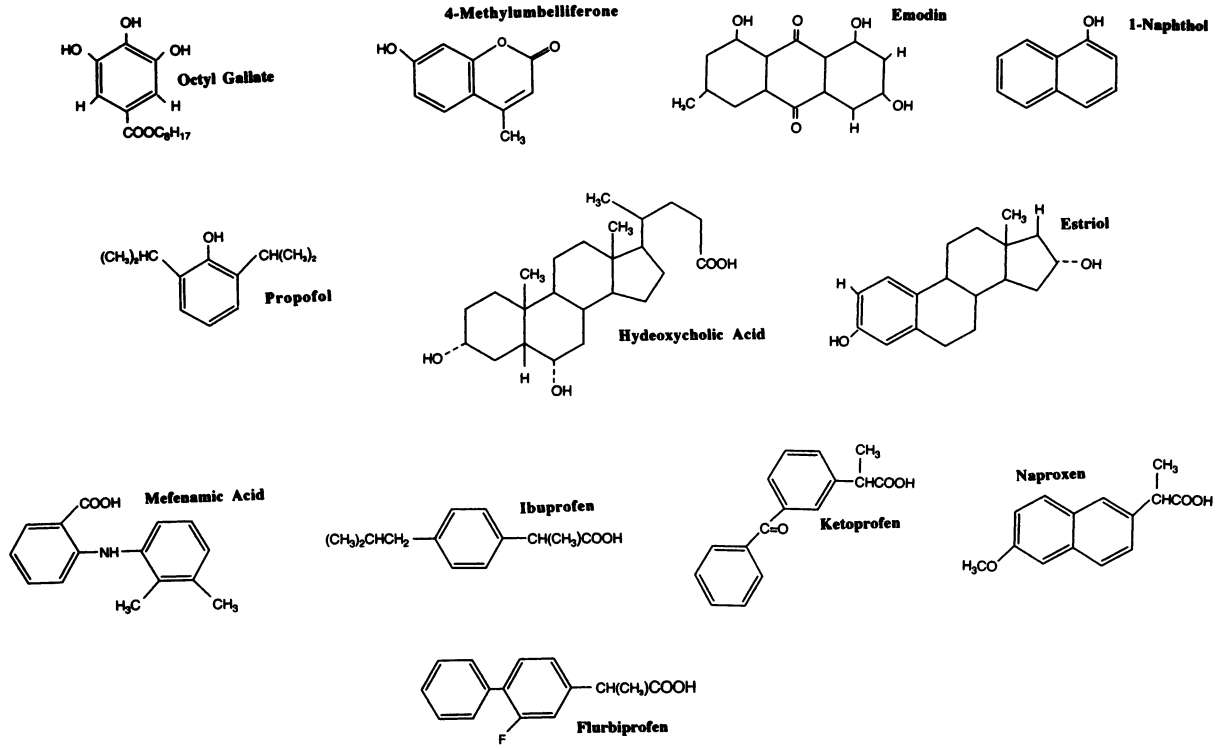

FIG. 2. Chemical structure of aglycone substrates.

The maximal levels of 1-naphthol UGT activity in human and rat kidney were similar although lower than the levels evident in both human and rat liver. Interestingly, optimal activation was achieved for both rat tissues at a similar detergent/protein ratio of D/P = 0.2. Analogous results were noted for the human hepatic and renal tissues (D/P = 0.35). Consistent with previous findings, in order to achieve maximal levels of UGT activity in tissues such as human kidney, the microsomal membrane must be disrupted [11, 29, 30]. Subsequent analyses of UGT activity in microsomal preparations were performed in the presence of optimally-activating concentrations of lubrol.

In vitro, the human kidney has the capacity to glucuronidate a number of chemically diverse compounds from simple phenols to steroids (Fig. 2). For the eleven substrates tested by TLC (Fig. 3) the activities apparently fell into three groups. The average kidney UGT activities towards octyl gallate, emodin and 4-methylumbelliferone were in the range of 10–15 nmol/min/mg protein. The average activities towards propofol, hyodeoxycholic acid and estriol were 3–5 nmol/min/mg protein and towards mefenamic acid, ibuprofen, ketoprofen and flurbiprofen the activities were 0.5–1.5 nmol/min/mg protein.

Bilirubin UGT activity was not detected in any of the human kidney samples analysed in agreement with previously published data indicating the complete absence of the major bilirubin UGT isoform, UGT1A1 from kidney [11, 14]. Control assays of bilirubin UGT activity in human liver and the cell line V79/UGT1A1 resulted in predicted levels of activity. In contrast, the endogenous substrates estriol and hyodeoxycholic acid were glucuronidated by human kidney.

The anaesthetic propofol (2,6-diisopropylphenol) was glucuronidated to a greater extent by four human kidney samples studied than two representative human liver samples. The average renal propofol UGT activity was 3 ± 0.71 nmol/min/mg protein compared with 0.7 ± 0.25 nmol/min/mg protein in liver. To date, the only cloned and expressed human UGT isoform capable of glucuronidating propofol *in vitro is* UGT1A8/9 [31].

Analysis with human kidney sample FK78 was limited to various NSAIDs due to limited tissue availability. The activities with these substrates were considerably lower than the four samples shown in Fig. 3. TLC analysis of FK78 microsomes were performed with only 125 μM UDPGA and this may have been a limiting factor (data not shown). Three further human kidney samples showed no significant 1-naphthol UGT activity, however these samples were studied by immunoblot analysis. These renal samples were generally of a poorer quality and this may account for the negligible levels of UGT activity.

Immunoblot Analysis of UGT Proteins in Human Kidney

Immunoblot analysis of human kidney samples using a polyclonal anti-rat UGT antibody was performed. Fewer bands were detected in human kidney (lanes 1–8) than in human liver, lanes 9 and 10 (Fig. 4). By comparison with standard molecular weight markers, the proteins recognised

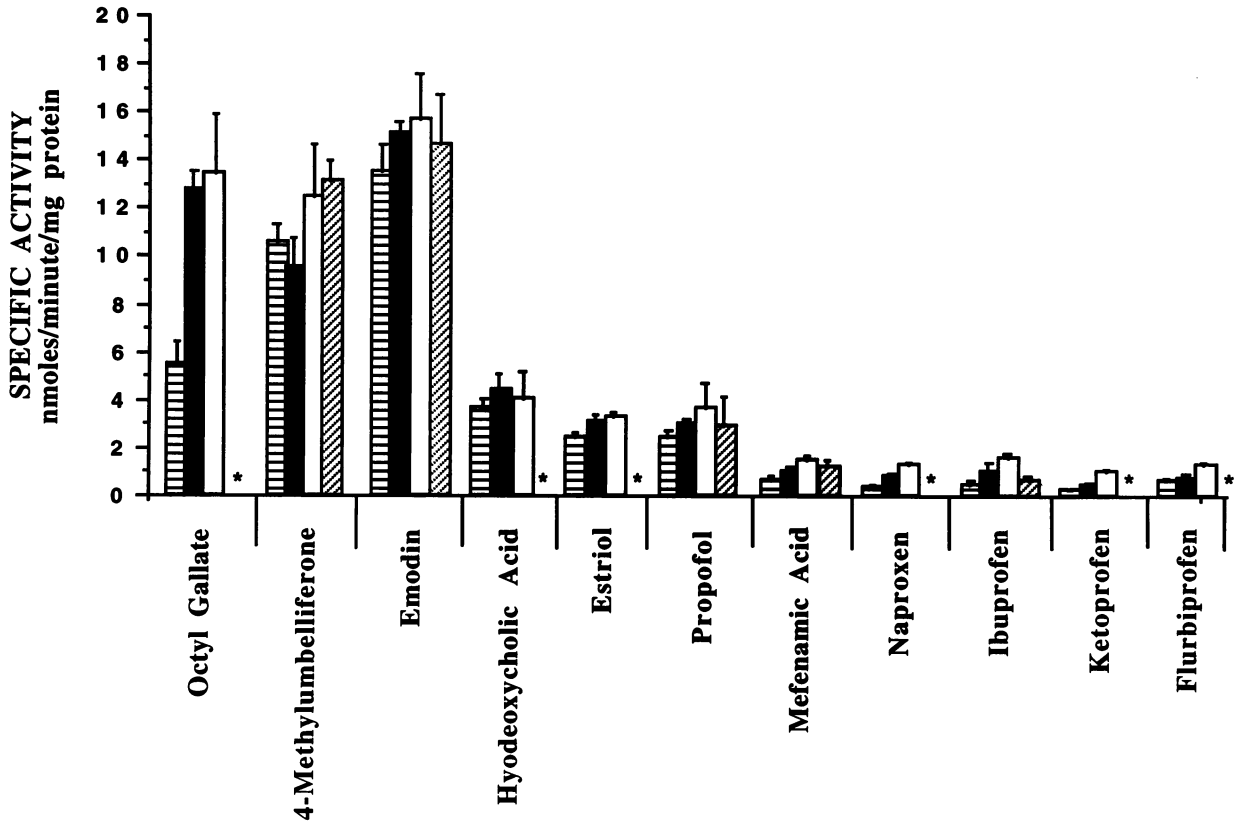

FIG. 3. Renal glucuronidation of various structurally diverse compounds. Human kidney microsomes were assayed towards aglycones using the TLC method outlined in the methods. Results are the average of three separate experiments ± SEM. The samples assayed were as follows; (▤) MK62; (■) MK63(a); (□) MK63(b); (▨) MK68. Assays indicated by the asterisks highlight aglycone substrates not tested with sample MK68.

by the anti-UGT antibody in human kidney were 52 and 54 kDa (Fig. 4).

Immunoblot data correlated with the enzymatic analysis, i.e. samples which did not exhibit significant UGT activity, namely lanes 5 (sample MK-1), 6 (sample MK-2) and 8 (sample MK45) gave little immunopositivity. Interestingly some variation was seen in the intensity of the two bands between different kidney samples. The levels of UGT activity were very similar between samples MK63 (a) and MK63 (b) and immunoblot analysis also indicated similar levels of expression for both bands, lanes 3 and 7, (Fig. 4). However, as with the enzymatic analysis, caution should be exercised when interpreting human data since environmental factors such as drug or xenobiotic exposure (see Table 1) may be significant.

DISCUSSION

Glucuronides of many drugs and xenobiotics are excreted in urine. We therefore aimed to characterise human renal UGT activities in order to assess the contribution of glucuronidation within the kidney to the elimination of aglycones, in addition to the excretion of conjugates formed by other tissues. Studies have shown that the kidney can significantly influence the overall glucuronidation of certain compounds e.g. diphenylacetic acid, probenecid and

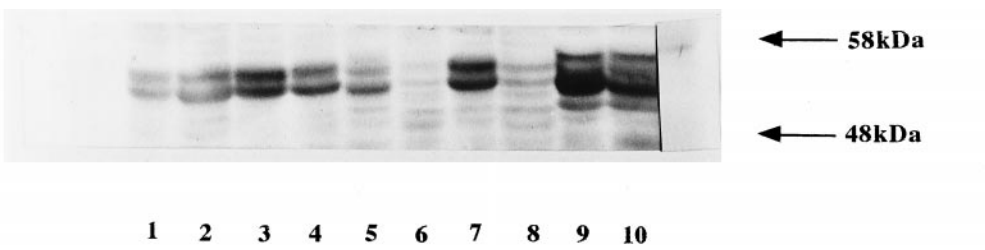

FIG. 4. Immunoblot analysis of human kidney microsomes using an anti-rat liver UGT antibody. Fifty μg of protein was loaded per lane on a 7.5% polyacrylamide gel. Immunoblotting was performed as outlined in Methods. Lane 1: FK47, lane 2: FK78, lane 3: MK63(a), lane 4: MK62, lane 5: MK1, lane 6: MK2, lane 7: MK63(b), lane 8: MK45, lanes 9 and 10, human liver microsomes.

naldixic acid [32, 33]. Renal dysfunction reduced the formation of diphenylacetic acid glucuronide in the rabbit by 22% [32].

Renal UGT activities are subject to complex regulation by chemical inducers. Carbon tetrachloride induced rat renal UGT activity in a parallel but less pronounced manner to that evident in the liver [34]. Salicylate administration has also been shown to induce UGT activity towards phenolic compounds in chicken kidney homogenate, but not in chicken liver homogenate [8, 35]. In addition, salicylate selectively enhanced renal glucuronide conjugation in the rat [35, 36]. The mechanism by which salicylate induces renal UGT activity is not understood, however it has important implications in terms of drug detoxication since there is worldwide use of this drug.

A rat phenolic UGT isoform (UGT1A6) was found to be constitutively expressed in liver and various extrahepatic tissues including kidney, testis, epididymis and ovary [37]. Indeed, the levels of this enzyme were considerably higher in the kidney than in the liver. Induction studies with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCCD) showed a marked increase in the liver enzyme (~10-fold) with only a modest 2-fold induction in the kidney form. These studies are consistent with tissue-specific regulation of the rat isoform UGT1A6 [37].

The data reported here further emphasises the potential importance of human kidney in drug biotransformation. The renal UGT activity was optimally released by the addition of the membrane perturbant lubrol as is the case in liver. We have shown that the kidney is capable of glucuronidating at least 12 chemically diverse compounds from simple phenols to steroids and NSAIDs.

The human UGT isoform UGT1A6 has a limited substrate specificity for the conjugation of simple phenols such as 1-naphthol and 4-nitrophenol [38]. The detection of human renal 1-naphthol UGT activity supports the presence of this isoform as implied by messenger RNA analysis [14] and as shown in rat kidney [37].

Bilirubin glucuronidation was not detected in any of the human kidney samples used in this study. The present study, performed on a larger number of samples confirms previous work on a single renal sample [14] highlighting the lack of bilirubin UGT activity (UGT1A1) in human kidney.

The human UGT isoforms UGT1A1 and UGT1A8/9 are known to glucuronidate the substituted phenol octyl gallate [23, 31]. The expression of cloned human UGT isoforms in cultured cells has shown the catalytic potential of UGT1A8/9 towards octyl gallate is approximately 40-fold greater than that of UGT1A1 [23]. The glucuronidation of octyl gallate was proposed to be the result of UGT1A8/9 activity since UGT1A1 is absent from human kidney. However, the contribution of another isoform cannot be ruled out.

The glucuronidation of propofol was also proposed to be due to the human UGT isoform UGT1A8/9 since no other isoform identified to date is capable of glucuronidating this compound [31]. The anthraquinone emodin was also glucuronidated by human kidney and is a substrate for a large spectrum of UGT isoforms including UGT1A8/9 [31]. Similarly 4-methylumbelliferone is also a broad spectrum substrate, therefore the activity towards these compounds cannot be associated with a specific UGT isoform in human kidney.

The endogenous compounds estriol and hyodeoxycholic acid are not glucuronidated by UGT1A8/9 [31] however the isoform UGT2B7 is capable of glucuronidating these compounds [39, 40] implying its presence in human kidney. Previous work has shown that the human liver UGT isoform UGT2B4 was also capable of glucuronidating hyodeoxycholic acid [41]. The use of a specific polyclonal antibody directed against human UGT2B4 demonstrated that UGT2B4 was exclusively located in the liver [42]. The results would suggest that UGT1A8/9 and UGT2B7 but not UGT1A1 are present in human kidney.

Propofol glucuronidation, proposed to be due to the UGT isoform UGT1A8/9 was consistently higher in human kidney then human liver. The diversity of human renal glucuronidation may result from the higher renal expression of UGT1A8/9 although this UGT cDNA was originally cloned from a human liver cDNA library [43]. Preliminary enzymatic, protein and RNA analysis suggested this glucuronosyltransferase was expressed at higher levels in human kidney [14]. Other groups have recently shown renal glucuronidation of propofol was significantly higher in human kidney compared with liver and small intestine [44].

Interestingly, UGT1A8/9 is the most promiscuous of all the human UGT isoforms cloned to date, in that it glucuronidates a wide range of chemically diverse substrates [31]. The presence of broad specificity UGTs in kidney could be beneficial in combating the barrage of foreign compounds to which this tissue is constantly exposed. Enzymes which accept chemically diverse compounds as substrates provide yet further flexibility within the human UGT enzyme family.

This report has attempted to address the question of renal glucuronidation, in particular the specific UGT isoforms that may be present in human kidney. Clearly, a number of UGT isoforms have yet to be characterised, however the use of specific substrates has begun to answer such questions. It is clear that the kidney is an organ which must be considered more fully in terms of drug biotransformation and in particular, glucuronidation.

This study was supported by grants from The Wellcome Trust. K. A. McGurk held a Wellcome Toxicology Prize Studentship.

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